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Studies on the Interaction of Water-Soluble Fullerols with BSA and the Effects of Metallic Ions

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ABSTRACT

The interaction of water-soluble C_{60} derived fullerols with bovine serum albumin (BSA) in physiological environment was studied in detail by the fluorescence method. Experiments showed that the interaction of fullerols with BAS is mainly in the manner of non-covalent hydrogen bond. Based on the measurements of fluorescence intensity, the apparent binding constant K and the binding site number n were obtained with K=4000 and n=1, and the energy transfer efficiency in the reaction is 0.63. Besides, the effects of metallic ions such as Cu^{2+}, Fe^{3+} and Cr(VI) on the interaction of fullerols with BSA were investigated. It was found that the effects of the metallic ions are quite different from each other. Low concentrations of Cu^{2+} can promote the interactions between fullerols and BSA, while high concentrations of Fe^{3+} or Cr(VI) favorite the interactions between fullerols and BSA.

INTRODUCTION

Fullerene C_{60} and its derivatives have attracted much interest due to their special structure, and novel chemical and physical functions[1]. However, their water-insoluble property has put much limit on their researches and applications, especially in biochemistry. The success in the synthesis of polyhydroxyl C_{60} derivative suggests the potential applications in biochemical and pharmaceutically related investigations[2-4]. In previous study, the fluorescence properties of fullerols and their interactions with various metallic ions were studied in detail[5]. Based on the study the fluorescence method was used to investigate the interaction between fullerols and BSA in physiological environment and the effects of various metallic ions, aimed at .understanding the interaction mechanism of fullerols with their biological surroundings.

EXPERIMENTAL

Apparatus and Reagents

All fluorescence measurements were carried out on a Hitachi RF-540 fluorescence photospectrometer. The wavelengthes of excitation and emission of fullerols were 340/440nm, respectively. Both of the excitation and emission slits were set as 10nm for providing the premium signals. IR spectra were recorded on a Model 1730 infrared photospectrometer, Perking-Elmer Co. and ^{1}HNMR spectra were obtained on a Hitachi R-24B Nuclear Magnetic

Resonance photospectrometer for the determination of fullerols structure.

C_{60} (99.9%) was purchased from Tri- Carbon Cluster Materials Co. Ltd., Wuhan University, China. BSA was purchased from Shanghai Lizhu Dongfeng Biotech Co. Ltd., China, with molecular weight of 65000. All chemicals used were analytical reagents. These reagents included the salts giving 3 kinds of ions, such as Cu^{2+}, Fe^{3+} and Cr(VI). The salts were $CuCl_2$, $FeCl_3$, and $K_2Cr_2O_7$, respectively. The water was secondary sub-boiled distilled water.

Procedure

Fullerols were prepared according to Ref.[7]. The final product was dark-brown and easily soluble in water. The structure of the product was then identified as $C_{60}(OH)_m$ by IR and ^{1}HNMR, where m, the number of –OH group, was 23-24.

An accurate amount of fullerols was weighed and prepared as stock solution of 1×10^{-3} mol/L using phosphate buffer solution with pH 7.4. The wavelengths of excitation and emission were 340/440nm, respectively. Both of the excitation and emission slits were set as 10nm for providing the premium signals. During experiments, an accurate volume of fullerols solution was injected by microinjector into BSA solution of 1×10^{-5} mol/L containing buffer solution and predetermined amount of metallic ion, and fluorescence spectrum was measured and recorded.

RESULTS AND DISCUSSIONS

Interaction of BSA with Fullerols

When 340nm is used as the excitation wavelength, fullerols can emit strong fluorescence at the wavelength of 440nm. In the previous study, the effects of pH value on fluorescence intensity have been presented[5]. The effect of pH value on fluorescence intensity can also be observed in this study. It is clear that the strongest fluorescence can only be observed in neutral solutions, which would lead to such a conclusion that fullerols in neutral molecular form are strong fluorescent substances. Fluorescence intensity becomes much weaker in both acidic and basic solutions. Therefore, in this research pH range of 6.5~7.5 was selected as the optimum acidity to carry out the bioactivity research of fullerols in physiological environment.

The excitation and emission spectra of fluorescence are given in Figure 1 and Figure 2. It can be seen that when 340nm is used as the excitation wavelength, fullerols can emit strong fluorescence at the wavelength of 440nm.

The fluorescence spectra of fullerols-BSA systems are shown in Figure 1. It can be seen that the fluorescence intensity at ~440nm increases markedly with the increase of the concentrations of fullerols. At the same time, the maximum emissions values at ~440nm have a

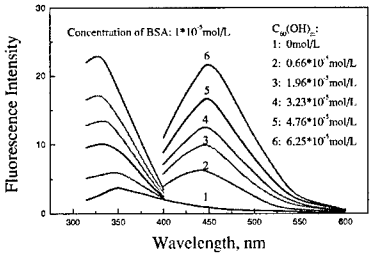

Figure 1 Fluorescence spectra of different concentrations of fullerols in the presence of BSA

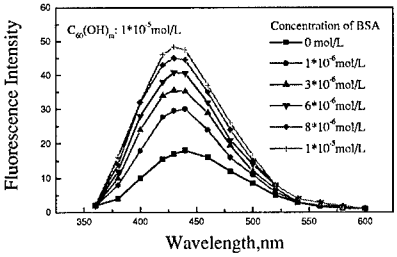

Figure 2 Fluorescence emission spectra of fullerols in the presence of different concentrations of BSA

gradual red-shift to 450nm, significant blue-shift can also be observed for excitation peaks from ~350nm to ~330nm. Based on the fact that there are only a strong absorption peak at 221nm and a weak absorption peak at 278nm, no absorption at 440nm for BSA, it can be suggested that there occurred some interaction between fullerols and BSA, which forms some kind of composite containing ground state and excited state. The absence of corresponding functional groups for chemical reaction between fullerols and BSA resulted in such a suggestion that the strong interaction between two molecules may be the hydrogen bridge bonding between the –OH in fullerols and the $-NH_2$ or –COOH in the peptide chains of BSA, which is in fact non-covalent.

The so-called bi-reciprocal method can be used to describe the interaction of fullerols with BSA[8]. From Figure 3 it can be seen that there exists very significant linear relationship in the plot of $(F-F_0)^{-1}$ against $C_{C60(OH)m}^{-1}$. The regression equation was found as follows

$$(F-F_0)^{-1} = (k'C_{BSA})^{-1} + (k'KC_{BSA})^{-1} C_{C60(OH)m}^{-1} \quad (1)$$

where F_0 is the fluorescence intensity of BSA without fullerols added in; F is the fluorescence intensity of BSA at presence of fullerols; C_{BSA} and $C_{C60(OH)m}$ are the concentrations of BSA and fullerols, respectively; k' is apparatus constant; and K is the apparent interaction constant. This relationship means that the combination ratio of fullerols and BSA is 1:1 with the K value of 4000 obtained from the slop and intercept of the plot in Figure 3.

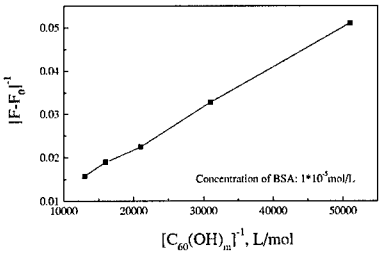

Figure 3 Bi-reciprocal plot for fullerols-BSA system

It was observed that the fluore- scence emission wavelength of BSA is 340nm (Figure 1), quite the same as that of the fluorescence excitation of fullerols. It is clear that the increase in the fluorescence intensity of fullerols with the increase of BSA concentrations originates in some energy transfer between them, as showin in Figure 2. The efficiency of energy transfer(E) is defined as following equation:

$$E=1-F_0/F \quad (2)$$

Where F_0 and F are the fluorescence intensities of fullerols in the absence and presence of BSA, respectively. It can be calculated that the E value is 0.63 when the concentrations of fullerols and BSA are 1:1.

The Effects of Cu^{2+}, Fe^{3+} and Cr(VI) on the Interactions of Fullerols with BSA

From previous study on the interaction of fullerols with metallic ions[5], It was found that Cu^{2+}, Fe^{3+} and Cr(VI) can quench the fluorescence of fullerols effectively. Here the three ions were introduced to investigate their effects on the interactions of fullerols with BSA.

Shown in Figure 4 are the fluorescence spectra of fullerols in the presence of Cu^{2+}, Fe^{3+} and Cr(VI) at a constant BSA concentration of 1×10^{-5} mol/L. In the case of Cu^{2+} or Fe^{3+}, with the increase of fullerols concentrations, the fluorescence intensity of BSA-M^{n+}-Fullerols increases and the emission peaks red-shifts gradually from 440nm to about 450nm, indicating the formation of more stable tri-component complex in both ground state and excited state among fullerols, BSA and Cu^{2+} or Fe^{3+} than bi-component complex between fullerols and BSA. In the case of Cr(VI), the little change in the shape and location of the peaks indicates that the formation of tri-component complex can only occur in excited state.

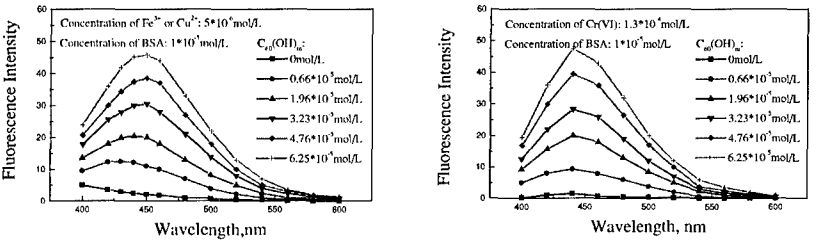

Figure 4 Fluorescence emission spectra of fullerols in the presence of BSA and in the presence of Cu^{2+}, Fe^{3+} or Cr(VI)

The K values at different concentrations of Cu^{2+}, Fe^{3+} and Cr(VI) are shown in Figure 5. An interesting phenomenon is that for all three curves a 'valley' occurs when the ratio of the concentrations of metallic ions and BSA is 1:1, indicating that competitive combination occurs between BSA and fullerols with the metallic ions. At this time, the presence of metallic ions doesn't show any promotion to the interaction of fullerols with BSA but show some role of inhibition. It can also be observed from Figure 5 that low concentrations of Cu^{2+} favorite the interaction of fullerols with BSA. When the ratio of Cu^{2+} to BSA is larger than 1:1, little apparent change can be observed,

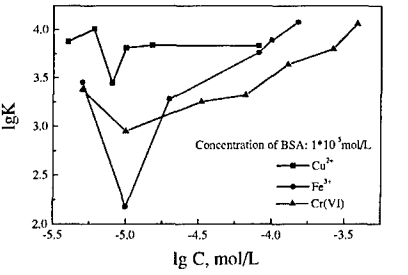

Figure 5 Binding constant K of BSA to fullerols in the presence of Cu^{2+}, Fe^{3+} or Cr(VI)

suggesting that at higher concentrations Cu^{2+} exhibit little effect on the interaction. In the case of Fe^{3+} and Cr(VI), their higher concentrations favorite the interaction of fullerols with BSA.

CONCLUSION

The interaction of water-soluble C_{60} derived fullerols with BSA in physiological environment was studied in detail. Experiments showed that the interaction of fullerols with BSA is mainly in the manner of non-covalent hydrogen bond. Based on the measurements of fluorescence intensity, the apparent binding constant K and the binding site number n were obtained with K=4000 and n=1, and the energy transfer efficiency in the reaction is 0.63. Besides, the effects of metallic ions such as Cu^{2+}, Fe^{3+} and Cr(VI) on the interaction of fullerols with BSA were investigated. It was found that the effects of the metallic ions are quite different from each other. Low concentrations of Cu^{2+} can promote the interactions between fullerols and BSA, while high concentrations of Fe^{3+} or Cr(VI) favorite the interactions between fullerols and BSA.

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